

X-Green II Double-Stranded DNA Quantification Kit (Upgraded Version)

X1372284

Storage Store at 2-8°C long term (20 months). Store in the dark.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

X-Green II is a product for fluorescent detection and quantification of dsDNA. This method is highly sensitive and widely applied in molecular biology workflows, including cDNA library construction, purification of DNA fragments for subcloning and related applications such as DNA quantification, product amplification and further primer detection.

The conventional method for DNA quantitation measures absorbance at 260 nm. Its major drawbacks include strong signal interference from nucleotides, single-stranded nucleic acids and proteins, as well as contaminants carried over during nucleic acid preparation. It cannot distinguish DNA from RNA and suffers from low sensitivity ($A_{260} = 0.1$ for 5 µg/mL dsDNA solution). Simple and convenient, the X-Green II quantitation method has become a standard test for residual DNA in biological products.

X-Green II emits fluorescence only upon binding to dsDNA, and the fluorescence intensity is proportional to DNA concentration. The upgraded X-Green II Double-stranded DNA Quantification Kit detects dsDNA within the range of 25 pg/mL to 1000 ng/mL with good linearity ($R^2 > 0.99$).

Kit Contents

X1372284	Components	200 T	2000 T	Storage
X1372284A	X-Green II	0.1 mL	1 mL	2-8 °C, protect from light
X1372284B	20×Buffer	2 × 1.25 mL	25 mL	2-8 °C, protect from light
X1372284C	dsDNA Standard (100 µg/mL)	0.1 mL	1 mL	2-8 °C, protect from light

Storage Conditions

Store at 2-8 °C protected from light. See outer packaging for shelf life. For long-term storage, 20×Buffer and dsDNA Standard can be stored at ≤ -20 °C.

Product Specifications

Ex/Em: 480/520 nm (when bound to dsDNA).

Protocol

Reagent Preparation

X-Green II is supplied as a 1 mL concentrate in organic solvent. Prepare 2× X-Green II Working Solution for assay: dilute Component A at a 1:200 ratio with 1×Buffer (20-fold dilution of Component B).

- For a final assay volume of 2 mL: to prepare working solution sufficient for 20 samples, add 100 μ L Component A into 19.9 mL 1×Buffer.
- For a final assay volume of 200 μ L: to prepare working solution sufficient for 20 samples, add 10 μ L Component A into 1.99 mL 1×Buffer.

The reagent readily adsorbs to glass surfaces; prepare the solution in plastic containers. Component A is light-sensitive and prone to degradation, so keep it protected from light. Prepare the solution and use it within several hours to obtain optimal assay results.

Assay Procedure

- Dilute Component B with sterile water to make 1×Buffer. For preparation of 50 mL 1×Buffer, add 2.5 mL Component B to 47.5 mL sterile water.
- Dilute the dsDNA Standard to generate the standard curve. Dilute Component C from 100 μ g/mL to 2 μ g/mL DNA stock using 1×Buffer. Example: take 40 μ L Component C and add to 1.96 mL sterile water. For low-range standard curves, further dilute the 2 μ g/mL DNA stock 40-fold to prepare a 50 ng/mL DNA stock solution, followed by serial dilution. Refer to Table 1 and Table 2 for detailed dilution schemes.

Table 1. Preparation of Wide-Range Standard Curve

Volume(μ L) of Buffer	Volume(μ L) of 2 μ g/mL DNA stock	Final DNA Concentration in X-Green II Assay
0	1000	1 μ g/mL
900	100	100 ng/mL
990	10	10 ng/mL
999	1	1 ng/mL
1000	0	blank

Table 2. Preparation of Low-Concentration Standard Curve

Volume(μ L) of Buffer	Volume(μ L) of 50 ng/mL DNA stock	Final DNA Concentration in X-Green II Assay
0	1000	25 ng/mL
900	100	2.5 ng/mL
990	10	250 pg/mL
999	1	25 pg/mL
1000	0	blank

- For each unknown sample, mix 1–10 μ L sample with 99–90 μ L 1 $\times$ Buffer, then transfer into a microplate well for detection.
- Prepare 2 $\times$ X-Green II Working Solution by diluting Component A 200-fold with 1 $\times$ Buffer. 100 μ L working solution is required for each standard and unknown sample. Calculate total reagent volume by multiplying 100 μ L by the total number of standards and unknown samples. X-Green II is photosensitive; perform thawing and dilution under light protection.
- Add 100 μ L diluted X-Green II Working Solution to each standard and sample well. Mix by pipetting up and down three times.
- Cover the microplate with aluminum foil and incubate at room temperature for 5 min.
- Read fluorescence signals. Take average values to plot the standard curve and calculate DNA concentration of unknown samples.

Notes

- Briefly centrifuge the product to collect liquid at the bottom of the tube before use, then proceed with experiments.
- Fluorescent dyes are subject to photobleaching. Minimize light exposure to slow down fluorescence quenching.
- Prepare X-Green II Working Solution freshly before use for optimal results.
- For personal safety and health, wear a lab coat and disposable gloves during operation.